

APPLICANTS: Vickers et al.
SERIAL NO: 10/664,639

DOCKET NO: CORE0027US

AMENDMENTS TO THE CLAIMS: This listing of claims replaces all prior versions and listings of claims in the instant patent application.

Listing of claims:

1. (Original) A method of identifying a multifunctional oligomeric compound to modulate expression of RNA comprising:

(a) contacting a target RNA with one or more double-stranded oligomeric compounds hybridizable to one or more target regions of said RNA and identifying double-stranded oligomeric compounds which inhibit target RNA levels by at least 50%;

(b) contacting the target RNA with an antisense strand of said modulating double-stranded oligomeric compound and determining whether the antisense strand inhibits target RNA levels by at least 50%; and

(c) identifying said inhibiting antisense strand and said inhibiting double-stranded oligomeric compound as multifunctional oligomeric compounds.

2. (Original) A multifunctional oligomeric compound identified according to claim 1.

3. (Original) A method of claim 1 wherein the multifunctional oligomeric compound inhibits target RNA levels by at least 80%.

4. (Original) The method of claim 1 wherein the target region is identified by a single-stranded oligomeric gene walk across the target RNA.

5. (Original) The method of claim 1 wherein the target region is identified by secondary structure analysis of the target RNA.

6. (Original) The method of claim 1 wherein said target region is at least a portion of an induced gene.

7. (Original) The method of claim 6 wherein the induced gene is CD54.

8. (Original) The method of claim 1 wherein said target region is at least a portion of a constitutive gene.

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9. (Original) The method of claim 1 wherein said target region is localized to the 3'UTR, the 5'UTR, an intron:exon boundary, an exon:exon boundary, a start region or a coding region of the RNA.

10-17. (Canceled)

18. (Original) The method of claim 1 wherein the oligomeric compound is an antisense oligonucleotide.

19. (Original) The method of claim 1 wherein the oligomeric compound has at least one modification of the base, sugar or internucleoside linkage.

20. (Original) The method of claim 1 wherein the oligomeric compound has a modification at the 2' position of at least one sugar.

21. (Original) The method of claim 1 wherein said oligomeric compound comprises at least four consecutive 2'-hydroxyl ribonucleosides and at least one modified nucleoside.

22. (Original) The method of claim 1 wherein said oligomeric compound is from about 12 to about 50 nucleotides in length.

23. (Original) The method of claim 1 wherein said oligomeric compound is from about 18 to about 25 nucleotides in length.

24. (Original) The method of claim 1 wherein said oligomeric compound comprises at least four consecutive 2'-hydroxyl ribonucleosides and at least one modified nucleoside; said modified nucleoside adapted to modulate at least one of; binding affinity or binding specificity of said oligomeric compound.

25. (Original) The method of claim 1 wherein the oligomeric compound is RNA.

26. (Original) The method of claim 1 wherein the oligomeric compound is a siRNA

27-28. (Canceled)

29. (Original) The method of claim 1 wherein the oligomeric compound is a gapmer.

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30. (Original) The method of claim 1 wherein the oligomeric compound comprises at least six consecutive nucleosides with 2' modifications.

31. (Original) The method of claim 1 wherein the oligomeric compound is a hemimer.

32. (Original) The method of claim 1 wherein the oligomeric compound comprises at least one phosphorothioate linkage.

33. (Original) The method of claim 1 wherein the oligomeric compound is a chimeric compound.

34. (Original) The method of claim 1 wherein the oligomeric compound comprises one or more chimeric regions.

35-49. (Canceled)

50. (Original) A method of selecting a single-stranded oligomeric compound comprising;

(a) contacting a target RNA with one or more double-stranded oligomeric compounds;

(b) identifying one or more double-stranded oligomeric compounds which inhibit target RNA levels by at least 50%; and

(c) selecting the strand of the identified double-stranded oligomeric compound which is complementary to the target RNA as the selected single-stranded oligomeric compound.

51. (Original) A method of selecting a double-stranded oligomeric compound comprising:

(a) contacting a target RNA with one or more single-stranded oligomeric compounds;

(b) identifying one or more single-stranded oligomeric compounds which inhibit target RNA levels by at least 50%; and

(c) hybridizing a complementary single-stranded oligomeric compound to said identified single-stranded oligomeric compound, yielding a double-stranded oligomeric compound as the selected double-stranded oligomeric compound.

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52-55. (Canceled)

56. (Previously presented) The oligomeric compound of claim 84 wherein said oligomeric compound inhibits RNA levels by at least 60% in both single-stranded and double-stranded forms.

57. (Previously presented) The oligomeric compound of claim 84 wherein the sequence homology is at least 90%.

58. (Previously presented) The oligomeric compound of claim 84 wherein the oligomeric compound has at least 2 mismatches as compared to the complement of the target RNA.

59. (Original) The oligomeric compound of claim 58 wherein the mismatches are internal or external base mismatches.

60. (Previously presented) The oligomeric compound of claim 84 wherein no more than two of the four 3'-most nucleotides of the oligomeric compound are mismatches.

61-62. (Canceled)

63. (Previously presented) The oligomeric compound of claim 84 wherein said oligomeric compound is targeted to the 3'UTR, the 5'UTR, an intron:exon boundary, an exon:exon boundary, a start region or a coding region of the RNA.

64-68. (Canceled)

69. (Previously presented) The oligomeric compound of claim 84 wherein said oligomeric compound has alternating linkages.

70. (Previously presented) The oligomeric compound of claim 84 wherein the oligomeric compound has alternating modifications.

71. (Previously presented) The oligomeric compound of claim 84 wherein every second nucleotide in the antisense strand of the double stranded oligomeric compound is modified.

72. (Original) The oligomeric compound of claim 71 wherein the first modified nucleotide is the 5'-most nucleotide of the oligomeric compound.

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73. (Original) The oligomeric compound of claim 71 wherein the modifications are 2' modifications.

74. (Original) The oligomeric compound of claim 71 wherein the modifications are one or more of 2'-O alkyl, 2'-O-methoxyethyl, 2'-methoxyethoxy, 2'-dimethylaminoxyethoxy, 2'-dimethylaminoethoxyethoxy, 2'-methoxy, 2'-aminopropoxy, 2'-allyl, 2'-O-allyl (2'-O-CH₂-CH=CH₂), or 2'-fluoro.

75. (Previously presented) The oligomeric compound of claim 84 wherein said oligomeric compound comprises:

a first segment;

a second segment; and,

a third segment comprising three or four nucleobases, said third portion

located between said first and second segments;

wherein said first and second segments each have at least one modified nucleobase.

76. (Canceled)

77. (Original) The oligomeric compound of claim 75 wherein said first and second segments each comprise at least one modified linkage/modification.

78. (Canceled)

79. (Previously presented) The oligomeric compound of claim 84 wherein said oligomeric compound hybridizes to at least a portion of the 3' UTR of said target RNA.

80. (Previously presented) The oligomeric compound of claim 84 wherein said oligomeric compound comprises at least four consecutive 2'-hydroxyl ribonucleosides and at least one modified nucleoside; said modified nucleoside adapted to modulate at least one of; binding affinity or binding specificity of said oligomeric compound.

81. (Previously presented) The oligomeric compound of claim 84 wherein said oligomeric compound comprises at least seven 2'-O-methyl substitutions at the 3'-terminus of the oligomeric compound.

82. (Previously presented) An oligomeric compound of claim 83 wherein the oligomeric compound has at least six mismatches as compared to the complement of the target RNA.

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83. (Previously presented) An oligomeric compound, 8-80 nucleobases in length, targeted to a target RNA, wherein said oligomeric compound specifically hybridizes said target RNA and wherein said oligomeric compound inhibits RNA levels by at least 50%.

84. (Previously presented) An oligomeric compound, 8-80 nucleobases in length targeted to a target RNA, wherein said oligomeric compound has at least 80% sequence homology to the complement of said target RNA and wherein said oligomeric compound inhibits RNA levels by at least 60%.

85. (New) A method of eliciting cleavage of a target mRNA in a cell, comprising contacting said cell with an antisense oligoribonucleotide 12 to 30 nucleobases in length targeted to said RNA, wherein said oligoribonucleotide comprises (i) no more than two mismatched nucleobases relative to said RNA and (ii) three 2'-O-methyl modifications on the 3' terminus.

86. (New) The method of claim 85 wherein said oligoribonucleotide further comprises a 5' terminal phosphate.

87. (New) The method of claim 85 wherein said oligoribonucleotide further comprises at least one phosphorothioate internucleoside linkage.

88. (New) The method of claim 87 wherein said oligoribonucleotide comprises a phosphorothioate internucleoside linkage at each position.

89. (New) The method of claim 85 wherein said oligoribonucleotide is 100% complementary to said RNA.

90. (New) A method of eliciting cleavage of a target RNA in a cell, comprising contacting said cell with an antisense oligoribonucleotide 12 to 30 nucleobases in length targeted to said RNA, wherein said oligoribonucleotide comprises (i) no more than two mismatched nucleobases relative to said target mRNA and (ii) 2'-fluoro modifications throughout the oligoribonucleotide.

91. (New) The method of claim 90 wherein said oligoribonucleotide further comprises a 5' terminal phosphate.

92. (New) The method of claim 90 wherein said oligoribonucleotide further comprises at least one phosphorothioate internucleoside linkage.

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93. (New) The method of claim 92 wherein said oligoribonucleotide comprises a phosphorothioate internucleoside linkage at each position.

94. (New) The method of claim 90 wherein said oligoribonucleotide is 100% complementary to said RNA.

95. (New) A method of eliciting cleavage of a target RNA in a cell, comprising contacting said cell with an antisense oligoribonucleotide 12 to 30 nucleobases in length targeted to said RNA, wherein said oligoribonucleotide comprises (i) no more than two mismatched nucleobases relative to said target mRNA and (ii) three to seven 2' modifications on the 3' terminus.

96. (New) The method of claim 95 wherein said oligoribonucleotide further comprises a 5' terminal phosphate.

97. (New) The method of claim 95 wherein said oligoribonucleotide further comprises at least one phosphorothioate internucleoside linkage.

98. (New) The method of claim 97 wherein said oligoribonucleotide comprises a phosphorothioate internucleoside linkage at each position.

99. (New) The method of claim 95 wherein said oligoribonucleotide is 100% complementary to said RNA.

100. (New) A single-stranded antisense oligoribonucleotide 12 to 30 nucleobases in length comprising three 2'-O-methyl modifications on the 3' terminus and at least one phosphorothioate internucleoside linkage.

101. (New) The oligoribonucleotide of claim 100 comprising phosphorothioate internucleoside linkages at each position.

102. (New) The oligoribonucleotide of claim 100 further comprising a 5' terminal phosphate.

103. (New) A single-stranded antisense oligoribonucleotide 12 to 30 nucleobases in length comprising 2'-fluoro modifications throughout the oligoribonucleotide and at least one phosphorothioate internucleoside linkage.

104. (New) The oligoribonucleotide of claim 103 comprising phosphorothioate internucleoside linkages at each position.

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105. (New) The oligoribonucleotide of claim 103 further comprising a 5' terminal phosphate.

106. (New) A single-stranded antisense oligoribonucleotide 12 to 30 nucleobases in length comprising three to seven 2' modifications on the 3' end and at least one phosphorothioate internucleoside linkage.

107. (New) The oligoribonucleotide of claim 106 comprising phosphorothioate internucleoside linkages at each position.